U.S. Patent No. 7,598,365 Attorney Docket: 58764.000062

Exhibit A U.S. Patent No. 7,598,365



US007598365B2

(12) United States Patent D'Halluin et al.

(10) Patent No.: US 7,598,365 B2 (45) Date of Patent: Oct. 6, 2009

(54) TARGETED DNA INSERTION IN PLANTS

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(*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 232 days.

(21) Appl. No.: 10/580,076

(22) PCT Filed: Nov. 17, 2004

(86) PCT No.: PCT/EP2004/013122

§ 371 (c)(1), (2), (4) Date:

May 18, 2006

(87) PCT Pub. No.: WO2005/049842

PCT Pub. Date: Jun. 2, 2005

(65) Prior Publication Data

US 2006/0282914 A1 Dec. 14, 2006

(30) Foreign Application Priority Data

(51)	Int. Cl.						
• /	C07H 21/04	(2006.01)					
	C12N 15/82	(2006.01)					
	C07K 14/00	(2006.01)					
	A01H 5/00	(2006.01)					

- (52) **U.S. Cl.** **536/23.2**; 536/23.4; 800/278; 800/320.1; 435/468; 435/419; 530/350

(56) References Cited

U.S. PATENT DOCUMENTS

5,474,896	A	*	12/1995	Dujon et al 43	35/6
5,689,052	A		11/1997	Brown et al.	
2002/0107214	Al	*	8/2002	Choulika et al 514	1/44

FOREIGN PATENT DOCUMENTS

CA	2 451 492	1/2003
EP	0 242 236 A1	10/1987
EP	0 242 246 A1	10/1987
EP	0 317 509 A2	5/1989
WO	WO 94/01560 A1	1/1994
WO	WO 94/17176 A1	8/1994
WO	WO 94/18313 A1	8/1994
WO	WO 94/26913	11/1994
WO	WO 95/09233 A1	4/1995
WO	WO 96/14408 A2	5/1996
WO	WO 00/46386 A2	8/2000
WO	WO 03/004659 A2	1/2003
WO	WO 03/054189 A2	7/2003

WO WO 03/080809 A2 10/2003

OTHER PUBLICATIONS

Guo et al., (2004) Protein Tolerance To Random Amino Acid Change P.N.A.S. 101 (25) 9205-9210).*

Lazar et al., (1988) Transforming Growth Factor A: Mutation Of Aspartic Acid 47 And Leucine 48 In Different Biological Activities. Molec. & Cell. Biol. 8(3)1247-52.*

Dujon, et al., 95.6% identicals to SEQ ID No. 1, found in the Issued Patents Database. Sequence No. 2 from 5474896-A US, issued on Dec. 12, 1995.*

Chilton et al. Targeted Integration of T-DNA into the Tobacco Genome at Double-stranded Breaks: New Insights on the MEchanism of T-DNA integration. (2003) Plant Physiology; vol. 133, pp. 956-965.*

Raikhel, N. Nuclear Targeting in Plants. (1992) Plant Physiology; vol. 100; pp. 1627-1632.*

Ashby, et al. "Ti Plasmid-Specified Chemotaxis of Agrobacterium tumefaciens C58C1 toward vir-Inducing Phenolic Compounds and Soluble Factors from Monocotyledonous and Dicotyledonous Plants", Journal of Bacteriology, vol. 170, No. 9, Sep. 1988, p. 4181-4187.

Bolton, et al., "Plant Phenolic Compounds Induce Expression of the *Agrobacterium tumefaciens* Loci Need for Virulence", Science, vol. 232, p. 983-985, 1986.

Chalfie, et al., "Green Fluorescent Protein as a Marker for Gene Expression", Science, vol. 263, p. 802-805, Feb. 11, 1994.

Chilton and Que, "Targeted Integration of T-DNA into the Tobacco Genome at Double-Stranded Breaks: New Insights on the Mechanism of T-DNA Integration", Plant Physiology, vol. 133, p. 956-965, Nov. 2003.

Choulika et al., "Induction of Homologous Recombination in Mammalian Chromosomes by Using the I-Scel System of Saccharomyces cerevisiae", Molecular and Cellular Biology, vol. 15, No. 4, p. 1968-1973, Apr. 1995.

Colleaux, et al., "Recognition and Cleavage Site of the Intron-Encoded *omega* Transposase", Proc. Natl. Acad. Sci. USA, vol. 85. p. 6022-6026, Aug. 1988.

Crameri, et al., "Improved Green Fluorescent Protein by Molecular Evolution Using DNA Shuffling", Nature Biotechnology, vol. 14, p. 315-319, Mar. 1996.

De Block, et al., "Engineering Herbicide Resistance in Plants by Expression of a Detoxifying Enzyme", The EMBO Journal, vol. 6, No. 9, p. 2513-2518, 1987.

Fennoy, et al., "Synonymous Codon Usage in Zea mays L. Nuclear genes is Varied by levels of C and G-ending Codons", Nucleic Acids Research, vol. 21, No. 23, p. 5294-5300, 1993.

Guivarc'h, et al., "Localization of Target Cells and Improvement of Agrobacterium-mediated Transformation Eficiency by Direct Acetosyringone Pretreatment of Carrot Root Discs", Protoplasma, vol. 174, p. 10-18, 1993.

Isalan, et al., "A Rapid, Generally Applicable Method to Engineer Zinc Fingers Illustrated by Targeting the HIV-1 Promoter", Nature Biotechnology, vol. 19, p. 656-660, Jul. 2001.

(Continued)

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(57) ABSTRACT

Methods and means are provided to improve targeted DNA insertion in plants using rare-cleaving "double stranded break" inducing enzymes. Also provided are improved I-SceI encoding nucleotide sequences.

20 Claims, 1 Drawing Sheet